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Modification by Light of 24-Hour Activity of White Rats

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Modification by Light of 24-Hour Activity of White Rats[1]

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Abstract. The 24-hour rhythm of running activity of white rats originates from a light-cycle and can be reversed by illuminating at night rather than by day. If continuous light is used, the activity phase of the rhythm remains 8-12 hours in length but shows a regular, constant and definite amount of alteration or delay in the time of starting so that the activity block travels around the clock. Behavior modification such as this should be called a photoperiodic response. In the present studies the photoperiodic data were: (1) the delay in time of starting activity for rats in continuous light was ¾ hour per day, and their activity traveled around the clock in 16 days; (2) during the progress of a reversal of the 24-hour solar light cycle, when light fell on the activity block there was a regular delay of 3 hours/day, only until the activity block again fell in darkness; (3) finally with a 16-hour light cycle of 8 hours of illumination and 8 hours of darkness, the delay was continuous at 1 hour a day. By raising rats with no cycle of nursing or other regular environmental influences, further evidence was obtained for the inheritance of what seems to be an activity rhythm of about 12 hours of activity and 12 hours of inactivity.

The 24-hour rhythm of running activity of white rats originates from a light-cycle and can be reversed by illuminating at night rather than by day. If continuous light is used, the activity phase of the rhythm remains 8-12 hours in length but shows a regular, constant and definite amount of alteration or delay in the time of starting so that the activity block travels around the clock. This observation was also made by Johnson (1939) for total activity of deer mice. The rate of change of time of activity is faster with an increase in the intensity of the light. When animals in continuous light are then in darkness the daily delay in time of starting ceases. Behavior modification such as this should be called a photoperiodic response. Other workers have found in day-active animals a reversed effect: an acceleration in continuous light (Barden 1942, lizard; Ashoff 1958, birds). The present paper describes two new applications of this photoperiodic effect, (1) during the progress of a reversal of the 24-hour solar light cycle, and (2) with a 16-hour light cycle of 8 hours of illumination and 8 hours darkness. Since these two responses are in addition to the continuous light response, there appears to be enough biological application to justify calling this photo-

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periodic response "the Johnson effect". Examples of these applications are described.

Methods

When measurements of 24-hour activity of rodents are made, the age, sex, nutritional state, and "activity history" of the animals must be taken into account. Levels of total activity and times of initiation can be affected by noise, accidental lack of food or water, insect bites, and above all, intensity of light. These factors all have been controlled in the following experiments. Activity was measured in revolutions by running wheel recorders developed by Welsh and illustrated and described in Farris and Griffith (1949). The accompanying photograph (Figure 1) of records from three rodent species (golden hamster, 13-

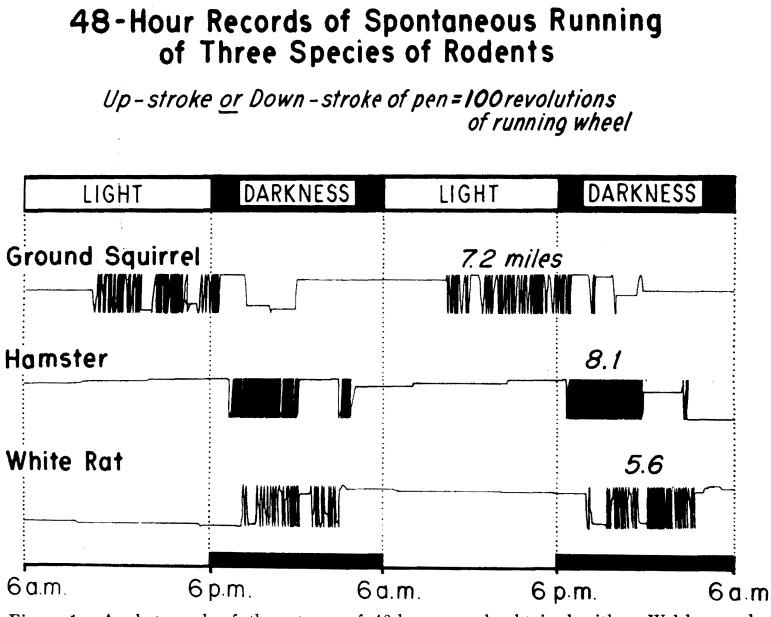

Figure 1. A photograph of three types of 48-hour records obtained with a Welsh recorder. The three species are 13-lined ground squirrel (*Citellus tridecemlineatus*), Syrian golden hamster, and Wistar white rat.

lined ground squirrel, Wistar white rat) shows how clearly it can be determined whether an animal is nocturnal, or whether it shows major activity in one or two blocks during the night. Four cages were used in each room in a suite of three sub-basement dark rooms in which no sounds from outside the suite could be detected by the human ear. The experiments were run at 24 $\pm$ 1° C., and there was no 24-hour humidity cycle. No experimental animal received any regular known environmental clues from outside the environmental chamber. Approximately 5.1 foot candles of illumination were used in all experiments. Graphical

analysis of all data was first done vertically in six blocks daily (Figure 2), because it was believed that other investigators have overlooked regular changes in time of starting activity by using horizontal graphing.

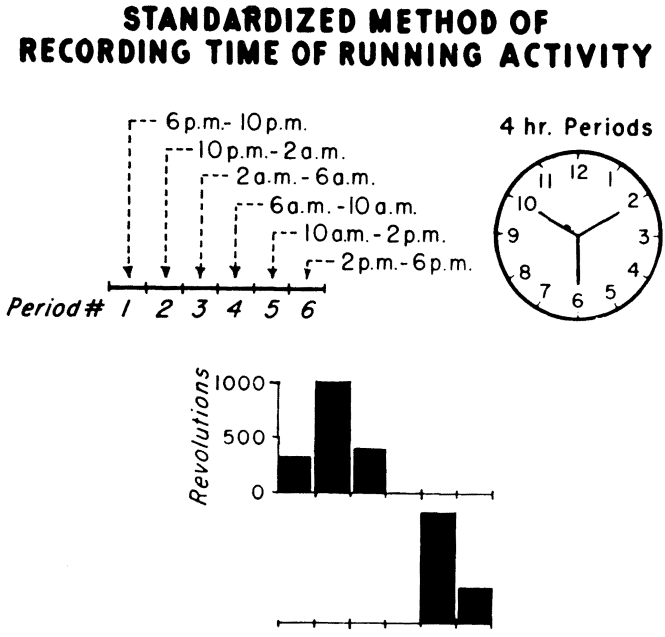

Figure 2. Throughout this study activity is recorded in six 4-hour periods each day. The above two bar graphs show the appearance when an activity peak (first bars) coming at midnight is delayed so that it comes at noon (second bars).

Results

Rats Raised Without Known Periodic Stimuli

Experiments were done to test the stability of the white rat "activity block," by trying to raise animals which have this block broken up (arrhythmic animals). One litter of six rats was raised without exposure to cycles of light and darkness and without the influence of a maternal 24-hour rhythm of activity. This was done to study the origin of the stimuli producing daily rhythmical activity. Young rodents raised in continuous darkness have always shown 24-hour rhythms of rest and activity. Since the influence of the mother has never been removed, their activity rhythm might have been acquired from the persistent activity rhythm of the mother. In the present experiment a maternal rhythm of activity was removed by exchanging a "foster" mother with the real mother at random times each day. Thus, the young nursed at any and most times of the day instead of mostly in daytime (the usual rat nursing regimen). By observation of the

bellies of the young it was determined that they did approximately the same amount of nursing from each mother. At the age of 104 days, the three best runners of the six rats were tested in activity cages in darkness. All three animals showed a clear pattern of activity varying from 8 to 12 hours, but with a daily activity rhythm of over 24 hours. The activity block was not broken up. This must be considered evidence that a rhythm of approximately 24 hours is inherited, but it must also be pointed out that this rhythm might have been conditioned during embryonic development in the mother. A comparison was made of the effects of continuous light and continuous darkness on these animals. The rate of change in time of activity was much greater with continuous light than with continuous darkness. These rates were nearly 2 hours per day in continuous light, and when continuous darkness followed, the rate changed abruptly to approximately 0.5 hours per day. According to terminology developed by Pittendrigh (1957), these animals raised without periodic stimuli, had a natural period (in darkness) varying from 24.5 hours a day to 25.0 hours a day.

Continuous Light

Rats were tested in continuous light after they were raised in a standard light cycle (12 hours light; 12 hours darkness daily). The resulting Johnson effect (Figure 3) was reported earlier (Folk,

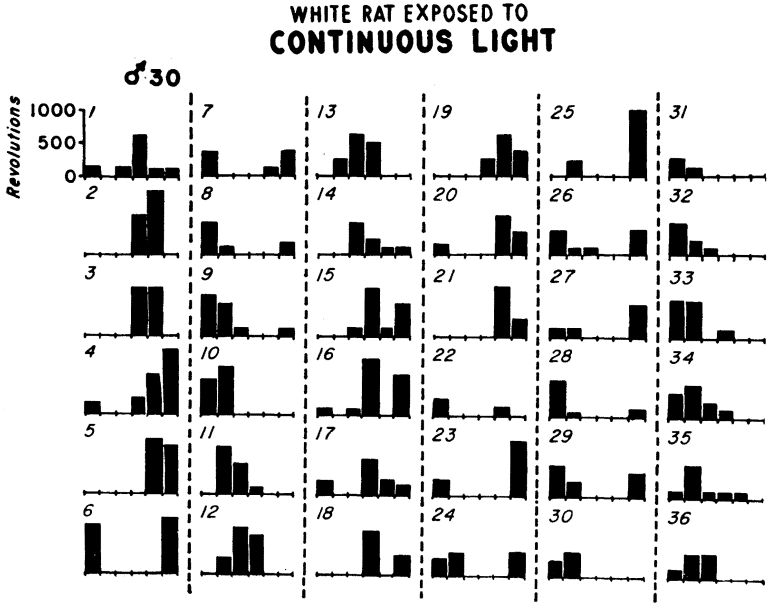

Figure 3. Animal number 30: A. Experimental period (continuous light): Day 1 to Day 16, showing a delay of 2 hours per day. B. Recovery control period (darkness); Day 17 to Day 36, showing a natural period of 25 hours a day.

1955). It is of interest that Brown (1956) was able to make use of this delay in time of starting activity in continuous light, in the white rat. As the activity block in his experiment traveled around the clock the amount of running activity did not remain constant but increased whenever the activity peak coincided with high tide, or with certain phases of the moon.

Additional data from four rats exposed to continuous light are now presented, expressed as average per cent of 24-hour activity in 6 hours (Figure 4). The first change in time of peak of ac-

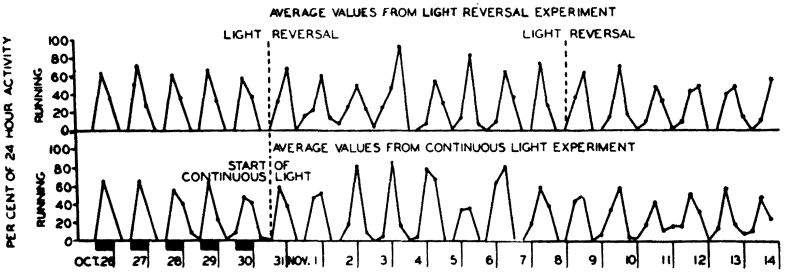

Figure 4. A comparison of the influence of continuous light upon running activity with that of a reversed 24-hour cycle of light and darkness (upper graph). The graph for the light-reversal experiment is the same as that in Figure 5. The data for the other graph were obtained by exposing four different male rats to continuous light. Results are presented together to demonstrate differences in rates of delay of activity.

tivity occurred in 2 days with continuous light. The next change took about 4 days; after 4 more days the activity peak had again been delayed for 6 hours. A final change by this amount would have moved the activity peak completely around the clock, but the experiment had to be terminated.

Reversal of 24-Hour Solar Light Cycle

A light reversal experiment was done with 4 male rats. When light fell upon the active animals, at first they continued regular running during the daylight period but with a gradual delay of 3 hours each day until the activity block again fell in darkness. This gradual and continuous delay appears to be due to the Johnson effect, in spite of the fact that continuous light was not part

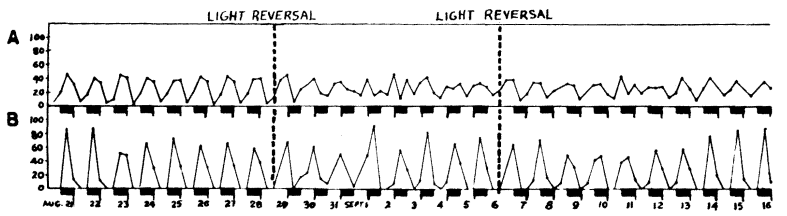

Figure 5. The effect upon total activity (running, Graph B; and feeding, Graph A), of reversal of the normal light cycle. The effect is gradual so that at first the animal runs during the daylight period. There is slight inhibition of running due to the reversal process.

of the experiment. Eight days were required for complete re-establishment of the activity pattern in darkness after the first reversal of the light cycle (Figure 5). On the ninth day the light cycle was again reversed. The fact that eight more days were again required this second time to change the pattern of activity by 12 hours is an indication that the new reversed running habit had become completely fixed. It could not be changed back to the original pattern in phase with the solar light cycle any more rapidly than it could be first brought in the reversed position. The effects of reversing a cycle of light and darkness can be compared with the effects of continuous light in Figure 4. In each experiment four male rats were used, in the same cages, with the same lights giving 5.1 foot candles. In spite of these facts the rate of change in time of activity was less rapid with continuous light than with a reversed 24-hour light cycle.

Sixteen Hour Light Cycle

An attempt was made with four male rats to convert their 24-hour rhythm to a 16-hour rhythm. After a control period, they were in continuous darkness for two weeks. This test showed the natural period of three of the rats to be nearly 24 hours,

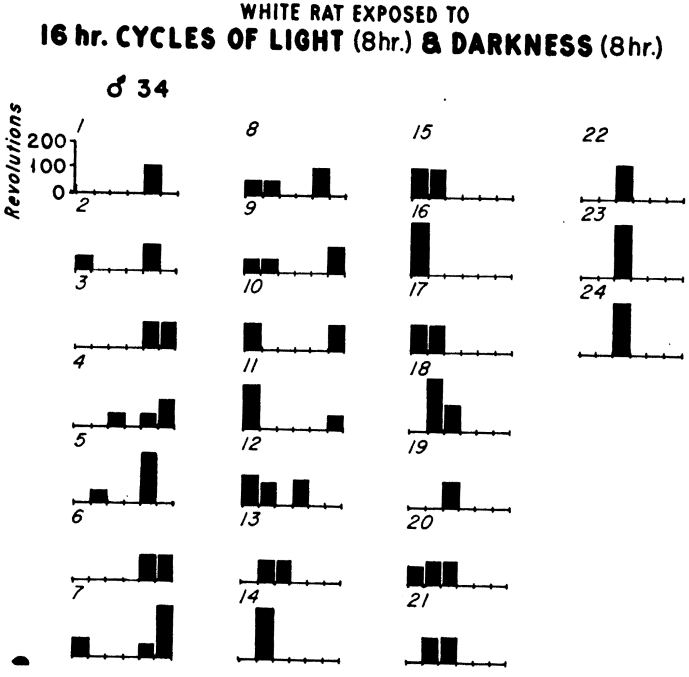

Figure 6. Animal number 34. A. Control period (darkness): Day 1 to Day 8, showing a natural period of just over 24 hours. B. Experimental period (16-hour cycle of light and darkness). The peak of activity is gradually delayed so it moves around the clock.

and of one rat to be 23 hours and 45 minutes. As soon as the 16-hour light cycle was begun, all four animals began to show a regular delay in time of starting of over one hour a day (Figure 6). A changing or dynamic activity state began, instead of a static one. This appears to be a typical Johnson effect. What happened to each animal was that it was illuminated during its activity period for about 8 hours during one 24-hour period, and for 4 hours during the next 24-hour period. This was continued on alternate days. The daily delay of starting activity was less than that caused by continuous light. The length of the active phase of the rhythm of these rats was as long at the end of this experiment as at the beginning. It is of interest that the delaying effect of an 8-hour period of light (perhaps one could say the driving effect of this period of light) caused the 12-hour period of activity to pass through each 8-hour period of darkness and yet to keep on being delayed. Part of the activity block was always completed by the animal with light shining on the cage. This suggests that if a 12-hour period of darkness were used, and 8 hours of light, success in breaking up the inherent 24-hour rhythm might be achieved.

Discussion

The photoperiodic effect first noted by Johnson (1939) appears to apply to rats in reversed and 16-hour light cycles. More experiments must be done with variable intensities of light; the equivalents of reversed light and the 8-hour light cycle should be tested in terms of continuous light. These experiments are difficult. It should be noted that with some individual animals continuous light decreases the level of total activity, and increasingly breaks up the block of activity as light intensities are increased. However, it is usually still possible to detect the time of starting of major activity.

The implications of the driving effect of continuous light can be found in behavior of animals in the polar zones, and in the design of laboratory experiments. If we reason from the experiments on rats and mice, then polar nocturnal animals in continuous light may have an activity peak which travels around the clock giving a rhythm of about 26 or 28 hours. This hypothesis should be checked by field work.

The influence of continuous light photoperiodicity on laboratory experiments can be illustrated by a recent paper by Sellar and Smart (1959). They injected insulin into mice at the same time of day, since mice respond differently in daytime (daymice) and night time (nightmice). One of nine variables studied was continuous light. There was an unexplained significant difference between the dosage effect on continuous-light mice and control mice,

and the authors noted that the animals which had been kept in continuous light were much more excitable than those kept in darkness. This probably means that the activity rhythm of the animals which had been kept in continuous light had shifted around the clock, and the authors were handling and injecting nightmice, not daymice.

In much of the interpretation of field and laboratory experiments, biologists should be aware that with continuous light and a reversed or 16-hour light cycle, rat and mouse activity shows a regular, constant, and definite amount of alteration or delay in the time of starting so that the activity block travels around the clock.

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